Journal of the American College of Cardiology
© 2002 by the American College of Cardiology Foundation
Published by Elsevier Science Inc.

Vol. 39, No. 9, 2002
ISSN 0735-1097/02/$22.00
PII S0735-1097(02)01792-8

Active Interstitial Remodeling: An Important Process in the Hibernating Human Myocardium

Nikolaos G. Frangogiannis, MD,*§ Sarah Shimoni, MD,†§ Su Min Chang, MD,†§ Guofeng Ren, PHD,*§ Oliver Dewald, MD,*§ Christine Gersch, PHD,*§ Kesavan Shan, MD,†§ Constandina Aggeli, MD,†§ Michael Reardon, MD,‡§ George V. Letsou, MD,‡§ Rafael Espada, MD,‡§ Mahesh Ramchandani, MD,‡§ Mark L. Entman, MD, FACC,*§ William A. Zoghbi, MD, FACC†§

Houston, Texas

OBJECTIVES The purpose of this study is to investigate the morphologic characteristics of the cardiac interstitium in the hibernating human myocardium and evaluate whether active remodeling is present and is an important determinant of functional recovery.

BACKGROUND Myocardial hibernation is associated with structural myocardial changes, which involve both the cardiomyocytes and the cardiac interstitium.

METHODS We evaluated 15 patients with coronary disease with two-dimensional echocardiography and thallium-201 (^{201}Tl) tomography before coronary bypass surgery. During surgery, transmural myocardial biopsies were performed guided by transesophageal echocardiography. Myocardial biopsies were stained immunohistochemically to investigate fibroblast phenotype and examine evidence of active remodeling in the heart.

RESULTS Among the 29 biopsied segments included in the study, 24 showed evidence of systolic dysfunction. The majority of dysfunctional segments (86.4%) were viable (^{201}Tl uptake ≥60%). After revascularization, 12 dysfunctional segments recovered function as assessed with an echocardiogram three months after bypass surgery. Interstitial fibroblasts expressing the embryonal isoform of smooth muscle myosin heavy chain (SMemb) were noted in dysfunctional segments, predominantly located in border areas adjacent to viable myocardial tissue. Segments with recovery had higher SMemb expression (0.46 ± 0.16% [n = 12] vs. 0.10 ± 0.02% [n = 12]; $p < 0.05$) and a higher ratio of alpha-smooth muscle actin to collagen (0.14 ± 0.026 [n = 12] vs. 0.07 ± 0.01 [n = 12]; $p < 0.05$) compared with segments without recovery, indicating fibroblast activation and higher cellularity of the fibrotic areas. In addition, interstitial deposition of the matricellular protein tenascin, a marker of active remodeling, was higher in hibernating segments than in segments with persistent dysfunction ($p < 0.05$), suggesting an active continuous fibrotic process. Multiple logistic regression demonstrated a significant independent association between SMemb expression and functional recovery ($p < 0.01$).

CONCLUSIONS Fibroblast activation and expression of SMemb and tenascin provide evidence of continuous remodeling in the cardiac interstitium of the hibernating myocardium, an important predictor of recovery of function after revascularization. (J Am Coll Cardiol 2002;39:1468–74)
© 2002 by the American College of Cardiology Foundation

The concept of myocardial hibernation was introduced 15 years ago to describe impaired left ventricular (LV) function that improves after revascularization (1,2). In the clinical context, it may be the result of repetitive ischemic episodes precipitated by increased demand in the setting of limited flow reserve or chronically low resting myocardial blood flow (3–5). Significant morphologic alterations involving both the myocytes and the cardiac interstitial space have been described in the hibernating myocardium (6–9). These structural changes are considered to be important factors responsible for the lack of immediate recovery of contractile function after restoration of blood flow (9). Previous studies have investigated the extensive structural alterations affecting cardiomyocytes of hibernating myocardial areas, which consist of depletion of contractile elements, glycogen accumulation and depletion of sarcoplasmic reticulum (10,11). Recently, the potential role of structural defects in the myocardial extracellular matrix has been suggested. Elsasser et al. (12) have found an enlarged extracellular space containing cellular debris, macrophages, fibroblasts and collagen fibrils, resulting in a significant degree of reparative fibrosis.

Fibroblasts have a crucial role in regulating extracellular matrix remodeling. In tissue fibrosis, fibroblastic cells differentiate into myofibroblasts, expressing smooth muscle cytoskeletal markers such as alpha-smooth muscle actin (α-SMAc). Activated myofibroblasts are dynamic regulators of the fibrotic process through the synthesis of extracellular matrix proteins and metalloproteinases. Recent studies in-

From the *Section of Cardiovascular Sciences and the †Section of Cardiology, Department of Medicine, and the ‡Department of Surgery, Baylor College of Medicine and the §DeBakey Heart Center, Houston, Texas. Supported by NIH Grant HL-42550, the DeBakey Heart Center, the John S. Dunn Sr. Trust Fund, the American Society of Echocardiography and a grant from the Methodist Hospital Foundation.

Manuscript received December 21, 2000; revised manuscript received January 24, 2002, accepted February 6, 2002.

Abbreviations and Acronyms

α-SMAc	= alpha-smooth muscle actin
LV	= left ventricular
SMemb	= embryonal isoform of smooth muscle myosin heavy chain
SPECT	= single photon emission tomography
TEE	= transesophageal echocardiography
TGF-β	= transforming growth factor-beta
^{201}Tl	= thallium-201

dicated expression of the embryonal isoform of smooth muscle myosin heavy chain (SMemb) by myofibroblasts in reperfused myocardial infarcts and in pressure-overloaded hearts (13). SMemb expression may serve as a marker of fibroblast activation, identifying cells involved in matrix remodeling. Furthermore, the matricellular protein tenascin has been shown to be a marker of actively remodeling tissues (14). Whether active remodeling in the interstitium is present in the hibernating myocardium and is an important determinant of recovery of function after revascularization has not been previously investigated. Accordingly, the present study evaluated the phenotypic characteristics of interstitial fibroblasts as well as SMemb and tenascin expression in the interstitium of hibernating human myocardium to examine their relation to recovery of myocardial function after revascularization.

METHODS

Patients scheduled for coronary artery bypass surgery who had known ischemic heart disease and LV systolic dysfunction at rest in the distribution of >1 coronary arteries with >70% stenosis were enrolled in this study. A transthoracic echocardiogram and thallium-201 single photon emission tomography (^{201}Tl SPECT) were performed two to seven days before surgery. During surgery, transmural biopsies were obtained from selected dysfunctional and normal myocardial segments, guided by transesophageal echocardiography (TEE) (15). Patients underwent transthoracic two-dimensional echocardiography three months after surgery to evaluate changes in regional function. The Institutional Review Board of Baylor College of Medicine approved the study protocol, and all patients signed informed consent before enrollment.

Echocardiographic studies. Imaging was performed in the standard parasternal and apical views with the patient in the left lateral position (Sonos 2500, 2.5- or 3.5-MHz transducer, Hewlett Packard, Andover, Massachusetts). Regional function was assessed according to the 16-segment model of the American Society of Echocardiography and graded from 1 to 5: 1 = normal, 2 = mild hypokinesia, 3 = severe hypokinesia, 4 = akinesia and 5 = dyskinesia (15). Ejection fraction was quantified with the multiple diameter method. The echocardiographic studies were interpreted without knowledge of the histopathologic data. Recovery of regional function was defined by improvement of ≥1 grades.

Rest-redistribution ^{201}Tl. Rest and 4-h redistribution ^{201}Tl SPECT scans were performed after intravenous administration of 3 mCi of ^{201}Tl before surgery as previously described (16). A large field-of-view rotating gamma camera with a high-resolution parallel-hole collimator was used. Thirty-two frames were acquired over a 180° arc (45° left posterior oblique to 45° left anterior oblique). The reconstructed images were oriented in the standard short axis, horizontal long axis and vertical long axis for interpretation and quantification of ^{201}Tl uptake by nuclear cardiologists unaware of all other data. Computerized polar maps of the three-dimensional myocardial radioactivity were generated. A 16-segment model comparable to that for echocardiography was used. Myocardial ^{201}Tl activity was determined with a region of interest 40 × 40 pixels (matrix 128 × 128). The activity in each segment was normalized to the segment with the highest uptake. A maximal uptake of ≥60% at rest or redistribution was considered indicative of viability, as previously demonstrated (16).

Transmural LV biopsies. Transmural myocardial biopsies were obtained with a 20-mm 14-gauge Tru-cut biopsy needle (Travenol Laboratories, Deerfield, Illinois) at the time of surgery, before cardioplegia. Biopsy was directed by TEE. Two segments were selected for each patient. For patients who had segments with normal systolic function, biopsies were acquired from one normal and one dysfunctional segment. For all other patients, two dysfunctional segments were biopsied. Segments with high likelihood for viability were targeted by avoiding very thin walls (<7 mm thickness) and echodense myocardium, usually indicative of a completed transmural infarction (17).

Immunohistochemistry and morphometry. Samples were fixed in B*5 fixative (18) and embedded in paraffin. Sections were stained with picrosirius red to identify areas of collagen deposition. Serial sections were stained immunohistochemically with the following antibodies: α-SMAc (Sigma, St. Louis, Missouri) (19), SM1 and SM2 (markers for mature smooth muscle cells), SMemb (Seikagaku America, Falmouth, Massachusetts) (20), tenascin (Chemicon International, Temecula, California) and vinculin (Serotec, Raleigh, North Carolina) (21). Staining was performed using a peroxidase based technique with the Vectastain ELITE kit (Vector Labs, Burlingame, California) and developed with diaminobenzidine + nickel (Vector), as previously described (18,22). Slides were counterstained with eosin and examined in a Zeiss microscope (Oberkochen, Germany).

Quantitative analysis. For quantitative analysis, each section was scanned at 200× magnification using a Leaf Lumina digital camera (Leaf Systems, Southboro, Massachusetts). The stained area for collagen, SMemb staining, tenascin and α-SMAc staining was quantitated using Zeiss Image software and expressed as the percentage of the total area of the section. In addition, the α-SMAc:collagen ratio, a marker of the cellularity of the cardiac interstitium, and

the tenascin:collagen ratio, an index of active remodeling, were calculated for each myocardial segment.

Statistical analysis. Data are presented as mean ± SEM. In order to examine intrapatient correlation of the segment data, we compared anterior and inferior or lateral segments from the same patients and found no correlation in any of the variables examined (collagen content: $r = 0.16$, $p = 0.63$; SMemb staining: $r = 0.08$, $p = 0.84$; tenascin: $r = -0.14$, $p = 0.71$; α-SMAc staining: $r = 0.34$, $p = 0.33$; change in wall motion score after revacularization: $r = 0.24$, $p = 0.79$). Thus, segment-by-segment analysis was performed as previously described (23). Unpaired *t* test was used to compare the pathologic variables between dysfunctional and nondysfunctional segments and segments with and without recovery of function after revascularization. Paired *t* test was used to compare the preoperative and postoperative ejection fraction. Collagen staining and α-SMAc expression were correlated with wall motion score using the Spearman coefficient. The same method was used for correlation studies between SMemb expression and the α-SMAc:collagen ratio or the tenascin:collagen ratio. Chi square analysis was used to examine the relation between SMemb or tenascin expression in the myocardium and recovery of function after revascularization. In addition, forward stepwise multiple logistic regression (Wald) was used to correlate recovery of function with ^{201}Tl uptake and the histomorphometric variables. GraphPad Instat version (GraphPad Software, San Diego, California) 3.1 and SSPS 7.0 (SPSS Inc., Chicago, Illinois) software were used for the statistical analysis. Significance was set at $p < 0.05$.

RESULTS

Patient population. Fifteen patients were enrolled in the study. There were 13 men and 2 women with a mean age of 62 years (range: 50 to 73 years). The mean LV ejection fraction was 29.4 ± 2%. Ten patients (67%) had hypertension, 9 (60%) were diabetic, 10 (67%) had angina pectoris and 8 (56%) had congestive heart failure. Thirteen patients underwent rest-redistribution thallium SPECT. Twenty-nine myocardial segments were biopsied and used for the study. One additional segment was excluded due to inadequate sampling. The average myocardial area of the biopsied samples used for quantitative analysis was 3.2 ± 0.45 mm^2 ($n = 29$). Five segments showed normal wall motion, 4 were mildly hypokinetic, 14 severely hypokinetic and 6 were akinetic. Mean myocardial ^{201}Tl uptake was 69.5 ± 2.4%. The vast majority of biopsied segments were viable (19 of 22 [86.4%] dysfunctional segments had an uptake ≥60%, and all except one had an uptake of ≥50%). All patients underwent complete revascularization without complications. Of the 24 dysfunctional segments, 12 segments recovered function after revascularization. The mean LV ejection fraction three months after revascularization was 38.6 ± 3.3% ($p < 0.001$, $n = 15$, compared with ejection fraction before revascularization).

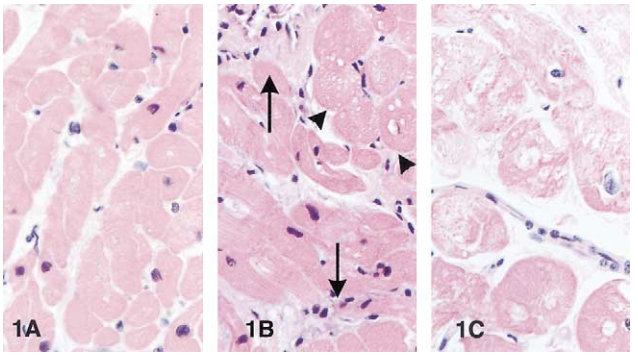

Figure 1. Structural alterations of the myocytes and the cardiac interstitium in dysfunctional myocardial segments. **(A)** Segment with preserved function showing relatively few morphologic abnormalities. **(B)** Segment with reversible dysfunction demonstrating a widened interstitial space **(arrows)**, with a relatively high cellular content, and myocyte degeneration with significant loss of contractile material **(arrowheads). (C)** Segments with persistent dysfunction after revascularization show more extensive pathologic changes, severe sarcomeric loss and significant extracellular matrix accumulation in the cardiac interstitium. All slides were stained with hematoxylin-eosin (400×).

Relation of collagen deposition to myocardial function. Cardiomyocytes in dysfunctional myocardial segments demonstrated significant structural abnormalities. Segments with persistent dysfunction after revascularization showed more extensive alterations, including sarcomere loss and disorganization of the contractile material (Fig. 1). Percent collagen content of the biopsies ranged between 6.32 and 72.4 (mean: 22.6 ± 2.99). A significant correlation was found between collagen percent staining and regional wall motion score ($r = 0.53$, $n = 29$; $p < 0.01$). Collagen staining was significantly higher in dysfunctional segments ($n = 24$) when compared with segments showing normal ($n = 5$) systolic function (24.99 ± 3.42 vs. 11.12 ± 1.11; $p < 0.001$).

Phenotypic characteristics of myofibroblasts in the myocardial interstitial space in dysfunctional segments versus normal. Immunohistochemical staining with antibodies to α-SMAc and the smooth muscle myosin isoforms SM1, SM2 and SMemb was used to identify myofibroblasts and smooth muscle cells in the myocardium. The α-SMAc immunoreactivity was localized in the vascular media and in pericytes and myofibroblasts, predominantly located in areas of fibrosis. Medial smooth muscle cells of myocardial arterioles expressed α-SMAc, SM1 and SM2 but not SMemb (Fig. 2). Interstitial cells expressing SMemb were noted in dysfunctional myocardial segments, predominantly located in border areas between fibrotic regions and areas with relatively preserved myocyte architecture (Fig. 3). These cells were negative for SM1 and SM2, both markers of mature smooth muscle cells. Percent staining with α-SMAc correlated well with collagen expression ($r = 0.64$, $n = 29$; $p = 0.0002$), reflecting the localization of myofibroblasts in areas of fibrosis. A trend towards higher α-SMAc expression was noted in dysfunctional myocardial segments compared with those with normal systolic func-

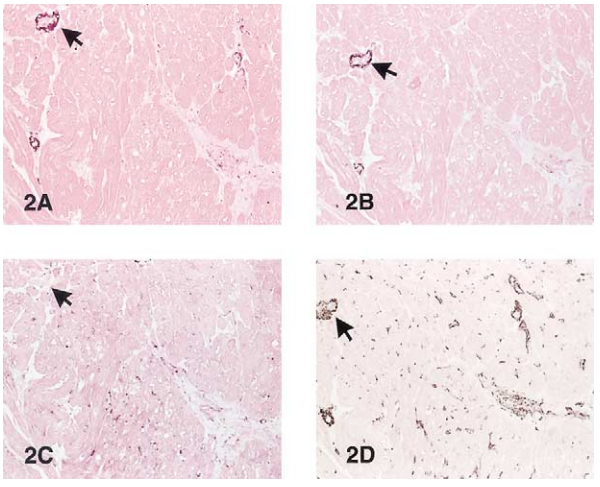

Figure 2. Smooth muscle cells and myofibroblasts in the dysfunctional human myocardium. Immunostaining of serial sections for SM1 **(A)**, SM2 **(B)**, markers of mature smooth muscle cells, the embryonic isoform of smooth muscle myosin heavy chain (SMemb) **(C),** and alpha-smooth muscle actin (α-SMAc) **(D).** Note that the myocardial arteriole **(arrows)** expresses SM1 **(A),** SM2 **(B)** and α-SMAc **(D)** but not SMemb **(C).** Expression of SMemb is noted in interstitial myofibroblasts **(C),** which are also positive for α-SMAc. Counterstained with eosin (100×).

tion (dysfunctional: 2.49 ± 0.5% [n = 24] vs. normal: 1.25 ± 0.47% [n = 5], p = 0.092).

Myofibroblasts also demonstrated expression of vinculin, a protein found in focal adhesion plaques, involved in cell attachment with extracellular matrix (Figs. 4C and 5C). Vinculin, also an important component of the cytoskeleton of myocytes, was localized in the sarcolemma and the intercalated discs. Cardiomyocytes with disorganized cytoplasmic expression of vinculin were found in dysfunctional myocardial segments (Fig. 5C).

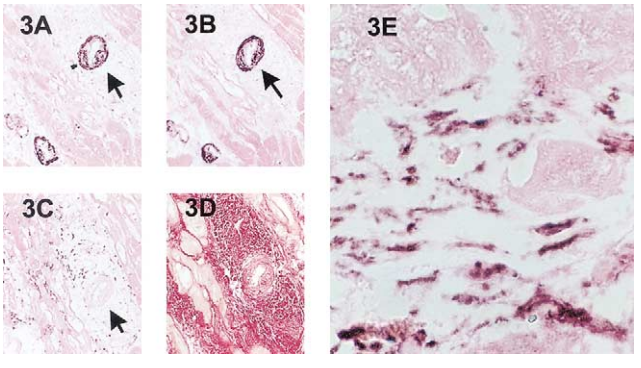

Figure 3. The embryonic isoform of smooth muscle myosin heavy chain (SMemb) positive cells are predominantly localized in border zone areas adjacent to viable myocytes. Serial sections from a hibernating myocardial segment were stained for SM1 **(A),** SM2 **(B),** SMemb **(C)** and collagen **(D).** Arteriolar smooth muscle cells **(arrows)** express SM1 and SM2 but not SMemb. In contrast, the interstitial SMemb positive cells **(C)** are negative for SM1 and SM2 and are predominantly located in border areas adjacent to viable myocytes. Areas with extensive collagen deposition show lower SMemb expression (100×). **(E)** High magnification image of an area from a hibernating myocardial segment stained for SMemb. Note that SMemb expression is localized in spindle-shaped interstitial cells (1,000×).

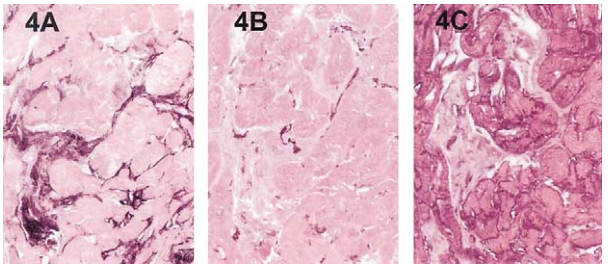

Figure 4. Immunohistochemistry for tenascin **(A),** alpha-smooth muscle actin (α-SMAc) **(B)** and vinculin **(C)** in the dysfunctional human myocardium from a segment with recovery of function after revascularization. Marked deposition of tenascin is noted in the cardiac interstitial space, suggesting an active continuous remodeling process. Interstitial myofibroblasts demonstrate expression of the focal adhesion protein vinculin **(C),** a factor promoting their attachment to the matrix. Note that vinculin immunoreactivity is also noted in the sarcolemma and the intercalated discs (200×). Counterstained with eosin.

High cellularity of the cardiac interstitium and expression of SMemb is associated with recovery of function after revascularization. Recovery of function occurred in 12 of 24 dysfunctional myocardial segments. Thallium uptake and quantitative morphometric parameters in segments with and without recovery are shown in Table 1. Thallium uptake was preserved in the majority of segments and was not significantly different in segments with and without recovery of function after revascularization (Table 1). In addition, percent collagen staining and percent α-SMAc staining were not significantly different in the two groups (collagen staining: recovery 20.2 ± 2.1% vs. no recovery: 29.8 ± 6.4%, p = 0.18 and α-SMAc staining: recovery of function, 1.87 ± 0.29% vs. no recovery 2.94 ± 0.87%, p = 0.26). However, segments with improved function demonstrated less advanced fibrotic changes, characterized by an interstitial space with a higher cellular content in relation to collagen deposition. This was reflected by the α-SMA:collagen ratio, which was significantly higher in segments with recovery (Table 1). Furthermore, segments with recovery showed significantly higher expression of SMemb when compared with segments demonstrating persistent dysfunction (Table 1). Segments with percent

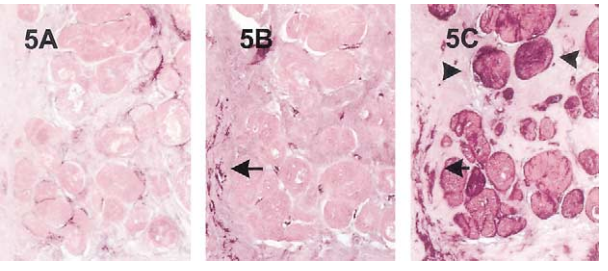

Figure 5. Immunohistochemistry for tenascin **(A),** alpha-smooth muscle actin (α-SMAc) **(B)** and vinculin **(C)** in the dysfunctional human myocardium from a segment with persistent dysfunction after revascularization. Tenascin immunoreactivity is present in the cardiac interstitium; however, its expression is low in comparison with the extent of fibrosis. In addition, vinculin-positive, α-SMAc expressing myofibroblasts are found in the fibrotic area **(arrow).** Note the presence of myocytes with extensive degenerative changes, demonstrating disorganized cytoplasmic expression of vinculin **(arrowheads).** Counterstained with eosin (200×).

Table 1. Comparison of ^{201}Tl Uptake and the Morphometric Variables in Segments With and Without Recovery of Function After Revascularization

Variables	Recovery	No Recovery	p Value
^{201}Tl uptake (%)	73.1 ± 2.7%	66.3 ± 4.3%	0.24
Collagen (%)	20.2 ± 2.1%	29.8 ± 6.4%	0.18
α-SMAc: collagen ratio	0.14 ± 0.026	0.07 ± 0.01	< 0.05
Tenascin: collagen ratio	0.074 ± 0.018	0.028 ± 0.01	< 0.05
SMemb (%)	0.46 ± 0.16%	0.10 ± 0.02%	< 0.05

α-SMAc = alpha-smooth muscle actin; SMemb = embryonal isoform of smooth muscle myosin heavy chain; ^{201}Tl = thallium-201.

SMemb staining >0.15% had significantly higher likelihood of recovery compared with those with <0.15% ($p = 0.012$).

Evidence for active remodeling in the cardiac interstitium from hibernating segments: expression of tenascin. To evaluate whether active matrix remodeling is present in the dysfunctional human myocardium, the expression of the matricellular protein tenascin was quantitated in the biopsied segments. There was low expression of tenascin in segments with normal function. In contrast, dysfunctional segments showed significantly higher expression of tenascin (dysfunctional: 1.29 ± 0.28% [n = 24] vs. normal: 0.32 ± 0.23% [n = 5]; $p = 0.016$), predominantly located in the cardiac interstitial space, indicating an active remodeling process (Fig. 4A). Tenascin staining was localized in the extracellular matrix, with minimal cytoplasmic expression by fibroblasts or smooth muscle cells. Importantly, segments with recovery of function demonstrated extensive tenascin staining in the interstitial space (Fig. 4A), whereas, in segments with persistent dysfunction, tenascin immunoreactivity was more limited in comparison with the extent of fibrosis (Fig. 5A). Segments with recovery of function showed a higher tenascin:collagen ratio than segments with persistent dysfunction (Table 1), indicating a more active remodeling process. Segments with a tenascin:collagen ratio >0.055 had a significantly higher likelihood of recovery than segments with a ratio <0.055 (odds ratio = 15.4, $p = 0.027$).

Histologic indicators of active remodeling are associated with recovery of function after revascularization. Using multiple logistic regression we found that percent SMemb staining ($p = 0.0098$) and the α-SMAc:collagen ratio ($p = 0.0215$) were significantly associated with recovery of function after revascularization. We identified a weaker association of the functional outcome with the tenascin:collagen ratio ($p = 0.093$), which did not attain statistical significance. Thallium uptake, which exceeded 60% in the majority of segments, was not an independent predictor of recovery ($p = 0.195$). Significant correlations were found between the two ratios and SMemb expression (α-SMAc: collagen ratio with SMemb $r = 0.483$, $p < 0.05$; tenascin: collagen ratio with SMemb $r = 0.634$, $p < 0.01$), reflecting the fact that all three morphometric variables represent indicators of interstitial space activity.

DISCUSSION

Chronic ischemic LV dysfunction is associated with profound structural alterations affecting both the cardiomyocytes and the interstitial space (24,25). There is a well-described loss of contractile material within the cardiomyocytes accompanied by a significant increase in extracellular matrix (26). Elsasser et al. (12) described the accumulation of collagen fibrils and fibronectin in the widened interstitial space, suggesting that the combination of cellular degeneration and fibrosis may determine the degree and speed of recovery of dysfunctional myocardial segments after bypass operation. They postulated that in the hibernating myocardium a self-perpetuating vicious cycle of tissue alterations may lead to progressive fibrosis and continuous cellular degeneration.

The present study examined the pathologic features of the cardiac interstitial space in patients with regional ischemic dysfunction. By excluding dysfunctional myocardial segments with an end-diastolic thickness <7 mm, we ensured that our study examines segments with viable myocardium and a substantial chance of recovery after revascularization (17). The viability of the biopsied myocardial segments was supported by ^{201}Tl SPECT, showing a ^{201}Tl uptake ≥60% in 86.4% (19 of 22) of the dysfunctional segments. The present study underlines the importance of the morphologic characteristics of the cardiac interstitium in determining recovery of function after revascularization. We report, for the first time, deposition of tenascin, a matricellular protein transiently expressed in actively remodeling tissues, in the interstitium of hibernating segments. In addition, we describe infiltration of the hibernating myocardium with phenotypically modulated myofibroblasts producing SMemb, a marker of activation. Our findings support the concept that ischemic myocardial dysfunction may be associated with a continuous, progressive fibrotic process, where activated interstitial fibroblasts may have a prominent role through the production of extracellular matrix components. The presence of active remodeling in the cardiac interstitium of the hibernating myocardium appears to be an important process in determining recovery of function after revascularization in segments with evidence of myocardial viability by thallium uptake and preserved myocardial thickness.

Fibroblasts in hibernating myocardium: α-SMAc expression and significance. Interstitial fibroblasts are responsible for myocardial collagen metabolism and may mediate systolic and diastolic ventricular dysfunction through the production of extracellular matrix proteins, matrix metalloptoteinases and growth factors (27–29). Fibroblast differentiation and activation is a prominent characteristic of wound healing and is associated with synthesis of α-SMAc and expression of a myofibroblast-like phenotype. Myofibroblasts are the main collagen-producing cells in myocardial infarcts (30) and may serve as a versatile cell population, assuming different phenotypes depending on the physiological needs (21). In the present study, dysfunctional myocar-

dial segments showed significant numbers of myofibroblasts expressing α-SMAc. Segments demonstrating recovery of function after revascularization had a higher α-SMAc: collagen ratio when compared with segments with persistent dysfunction (Table 1). This reflects a more cellular interstitial space in hibernating myocardial tissue. In contrast, segments without recovery showed extensive extracellular matrix deposition associated with a relatively low expression of α-SMAc. This finding may be explained by the limited life span of myofibroblasts in fibrotic tissues; during wound healing myofibroblasts disappear by means of apoptosis, and granulation tissue gradually transforms into scar tissue (31). Thus, segments without recovery of function may represent the late stages of a continuous process of interstitial fibrosis, associated with a less cellular environment.

Fibroblasts in hibernating myocardium: SMemb expression and significance. The SMemb is a nonmuscle myosin heavy chain isoform, predominantly present in the fetal aorta, whose expression is upregulated in immature dedifferentiated intimal cells (32). Recently, SMemb induction was noted in activated cardiac myofibroblasts from pressure overloaded rat hearts (13) and in the border zone of healing myocardial infarcts (21). Myofibroblast differentiation and SMemb expression can be induced by stimulation with transforming growth factor-beta (TGF-β) (13), a cytokine known to be upregulated in the hibernating myocardium (33). To date, whether SMemb is expressed in hibernating myocardium has not been previously investigated. In the present study, a subpopulation of myofibroblasts expressed SMemb and was predominantly located in border areas between fibrotic regions and areas with preserved myocyte architecture (Figs. 2 and 3). The SMemb expression was significantly higher in segments that exhibited recovery of function after revascularization (Table 1). This may again reflect an earlier stage of the fibrotic process associated with fibroblast activation. In addition, in a multiple logistic regression model, SMemb expression was significantly and independently associated with the functional outcome after revascularization. This finding underlines the association between an active interstitial space and functional recovery.

Tenascin deposition in hibernating myocardium. Tenascin is a multifunctional extracellular matrix glycoprotein that is exquisitely regulated during embryonic development and in adult tissue remodeling (14). Tenascin belongs to a class of matrix proteins, termed matricellular proteins, that function as modulators of cell-matrix interactions (34). Matricellular proteins may regulate tissue remodeling through promotion of the "de-adhesive state," an intermediate state of cellular adhesiveness, which may facilitate repair and adaptation (35). Tenascin is not normally expressed in the adult heart but reappears in a variety of pathologic conditions (36) such as dilated cardiomyopathy and myocardial infarction (37,38). During the acute phase of myocardial infarction, tenascin is synthesized and deposited in the border zone, possibly facilitating tissue reorganization (37); however, its expression almost disappears two weeks after a nonreperfused myocardial infarction. Our study demonstrated persistent interstitial deposition of tenascin in the dysfunctional human myocardium, suggesting an active continuous process of matrix remodeling. The tenascin:collagen ratio, a marker of tenascin deposition in the cardiac interstitium, was significantly higher in segments with recovery (Table 1), suggesting the presence of an active remodeling process in hibernating segments. These findings support the concept that ischemic cardiomyopathy represents a dynamic process associated with continuous deposition of extracellular matrix, leading to a state of extensive fibrosis, potentially associated with a lower chance of functional recovery.

Potential implications of the findings for the pathophysiology of myocardial hibernation. In the absence of an acute coronary event, ischemic contractile dysfunction may be a chronically progressive condition. We propose the following pathophysiologic process that involves the interstitium in hibernating myocardium based on previous data and observations from the present study. In the early stages, ischemia-induced signals, such as TGF-β synthesis and release, may lead to fibroblast accumulation in the interstitial space. Accumulating fibroblasts may undergo phenotypic changes, such as the expression of α-SMAc and SMemb. Interstitial expression of the matricellular protein tenascin may have a role in modulating cellular adhesion and cytokinesis, promoting matrix remodeling. At a later stage, and despite the presence of myocardial viability (preserved thickness and ^{201}Tl uptake), the cardiac interstitium becomes a less cellular environment, dominated by extracellular matrix accumulation and showing lower levels of inflammatory activity. This stage is associated with a lower likelihood of functional recovery, further supporting the need for early revascularization in patients with suspected myocardial hibernation.

Acknowledgments

The authors thank Alida Evans and Stephanie Butcher for their outstanding technical assistance and Concepcion Mata and Sharon Malinowski for their expert secretarial assistance in preparing the manuscript.

Reprint requests and correspondence: Dr. Nikolaos G. Frangogiannis, Section of Cardiovascular Sciences, Department of Medicine, Baylor College of Medicine, One Baylor Plaza M/S F-602, Houston, Texas 77030. E-mail: ngf@bcm.tmc.edu.

REFERENCES

1. Rahimtoola SH. A perspective on the three large multicenter randomized clinical trials of coronary bypass surgery for chronic stable angina. Circulation 1985;72:V123–35.
2. Braunwald E, Rutherford JD. Reversible ischemic left ventricular dysfunction: evidence for the "hibernating myocardium". J Am Coll Cardiol 1986;8:1467–70.
3. Shivalkar B, Flameng W, Szilard M, Pislaru S, Borgers M, Vanhaecke J. Repeated stunning precedes myocardial hibernation in progressive multiple coronary artery obstruction. J Am Coll Cardiol 1999;34: 2126–36.

4. Bolli R. Myocardial "stunning" in man. Circulation 1992;86:1671–91.
5. Fallavollita JA, Logue M, Canty JM, Jr. Stability of hibernating myocardium in pigs with a chronic left anterior descending coronary artery stenosis: absence of progressive fibrosis in the setting of stable reductions in flow function and coronary flow reserve. J Am Coll Cardiol 2001;37:1989–95.
6. Chen C, Ma L, Linfert DR, et al. Myocardial cell death and apoptosis in hibernating myocardium. J Am Coll Cardiol 1997;30:1407–12.
7. Heusch G. Hibernating myocardium. Physiol Rev 1998;78:1055–85.
8. Schwarz ER, Schaper J, vom Dahl J, et al. Myocyte degeneration and cell death in hibernating human myocardium. J Am Coll Cardiol 1996;27:1577–85.
9. Ausma J, Cleutjens J, Thone F, Flameng W, Ramaekers F, Borgers M. Chronic hibernating myocardium: interstitial changes. Mol Cell Biochem 1995;147:35–42.
10. Schwarz ER, Schoendube FA, Kostin S, et al. Prolonged myocardial hibernation exacerbates cardiomyocyte degeneration and impairs recovery of function after revascularization. J Am Coll Cardiol 1998;31: 1018–26.
11. Maes AF, Borgers M, Flameng W, et al. Assessment of myocardial viability in chronic coronary artery disease using technetium-99m sestamibi SPECT: correlation with histologic and positron emission tomographic studies and functional follow-up. J Am Coll Cardiol 1997;29:62–8.
12. Elsasser A, Schlepper M, Zimmermann R, et al. The extracellular matrix in hibernating myocardium—a significant factor causing structural defects and cardiac dysfunction. Mol Cell Biochem 1998;186: 147–58.
13. Shiojima I, Aikawa M, Suzuki J, Yazaki Y, Nagai R. Embryonic smooth muscle myosin heavy chain SMemb is expressed in pressure-overloaded cardiac fibroblasts. Jpn Heart J 1999;40:803–18.
14. Jones PL, Jones FS. Tenascin-C in development and disease: gene regulation and cell function. Matrix Biol 2000;19:581–96.
15. Nagueh SF, Mikati I, Weilbaecher D, et al. Relation of the contractile reserve of hibernating myocardium to myocardial structure in humans. Circulation 1999;100:490–6.
16. Qureshi U, Nagueh SF, Afridi I, et al. Dobutamine echocardiography and quantitative rest-redistribution ^{201}Tl tomography in myocardial hibernation: relation of contractile reserve to ^{201}Tl uptake and comparative prediction of recovery of function. Circulation 1997;95:626–35.
17. Cwajg JM, Cwajg E, Nagueh SF, et al. End-diastolic wall thickness as a predictor of recovery of function in myocardial hibernation: relation to rest-redistribution T1-201 tomography and dobutamine stress echocardiography. J Am Coll Cardiol 2000;35:1152–61.
18. Frangogiannis NG, Mendoza LH, Lindsey ML, et al. IL-10 is induced in the reperfused myocardium and may modulate the reaction to injury. J Immunol 2000;165:2798–808.
19. Frangogiannis NG, Perrard JL, Mendoza LH, et al. Stem cell factor induction is associated with mast cell accumulation after canine myocardial ischemia and reperfusion. Circulation 1998;98:687–98.
20. Aikawa M, Yamaguchi H, Yazaki Y, Nagai R. Smooth muscle phenotypes in developing and atherosclerotic human arteries demonstrated by myosin expression. J Atheroscler Thromb 1995;2:14–23.
21. Frangogiannis NG, Michael LH, Entman ML. Myofibroblasts in reperfused myocardial infarcts express the embryonic form of smooth muscle myosin heavy chain (SMemb). Cardiovasc Res 2000;48:89–100.
22. Frangogiannis NG, Lindsey ML, Michael LH, et al. Resident cardiac mast cells degranulate and release preformed TNF-alpha, initiating the cytokine cascade in experimental canine myocardial ischemia/reperfusion. Circulation 1998;98:699–710.
23. Sawada S, Elsner G, Segar DS, et al. Evaluation of patterns of perfusion and metabolism in dobutamine-responsive myocardium. J Am Coll Cardiol 1997;29:55–61.
24. Vanoverschelde JL, Wijns W, Depre C, et al. Mechanisms of chronic regional postischemic dysfunction in humans: new insights from the study of noninfarcted collateral-dependent myocardium. Circulation 1993;87:1513–23.
25. Vanoverschelde JL, Wijns W, Borgers M, et al. Chronic myocardial hibernation in humans: from bedside to bench. Circulation 1997;95: 1961–71.
26. Depre C, Vanoverschelde JL, Melin JA, et al. Structural and metabolic correlates of the reversibility of chronic left ventricular ischemic dysfunction in humans. Am J Physiol 1995;268:H1265–75.
27. Gray MO, Long CS, Kalinyak JE, Li HT, Karliner JS. Angiotensin II stimulates cardiac myocyte hypertrophy via paracrine release of TGF-beta 1 and endothelin-1 from fibroblasts. Cardiovasc Res 1998;40: 352–62.
28. Weber KT, Brilla CG, Janicki JS. Myocardial fibrosis: functional significance and regulatory factors. Cardiovasc Res 1993;27:341–8.
29. Weber KT. Cardiac interstitium in health and disease: the fibrillar collagen network. J Am Coll Cardiol 1989;13:1637–52.
30. Cleutjens JP, Verluyten MJ, Smiths JF, Daemen MJ. Collagen remodeling after myocardial infarction in the rat heart. Am J Pathol 1995;147:325–38.
31. Gabbiani G. The cellular derivation and the life span of the myofibroblast. Pathol Res Pract 1996;192:708–11.
32. Aikawa M, Sakomura Y, Ueda M, et al. Redifferentiation of smooth muscle cells after coronary angioplasty determined via myosin heavy chain expression. Circulation 1997;96:82–90.
33. Elsasser A, Decker E, Kostin S, et al. A self-perpetuating vicious cycle of tissue damage in human hibernating myocardium. Mol Cell Biochem 2000;213:17–28.
34. Sage EH. Regulation of interactions between cells and extracellular matrix: a command performance on several stages. J Clin Invest 2001;107:781–3.
35. Murphy-Ullrich JE. The de-adhesive activity of matricellular proteins: is intermediate cell adhesion an adaptive state? J Clin Invest 2001;107: 785–90.
36. Yamamoto K, Dang QN, Kennedy SP, Osathanondh R, Kelly RA, Lee RT. Induction of tenascin-C in cardiac myocytes by mechanical deformation: role of reactive oxygen species. J Biol Chem 1999;274: 21840–6.
37. Imanaka-Yoshida K, Hiroe M, Nishikawa T, et al. Tenascin-c modulates adhesion of cardiomyocytes to extracellular matrix during tissue remodeling after myocardial infarction. Lab Invest 2001;81: 1015–24.
38. Willems IE, Arends JW, Daemen MJ. Tenascin and fibronectin expression in healing human myocardial scars. J Pathol 1996;179: 321–5.